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Comparative effects of butyrate, herbal extract, and yeast on growth performance, serum parameters, organ weight, and ileum morphology in broilers

Muhittin Zengin[1,2*], Ergün Demir[1], Mustafa Usta[3], Oğuz Koray Bacaksız[1], Musa Karaman[3] and Mehmet Ali Azman[1]

*Correspondence:
Muhittin Zengin
mzengin@balikesir.edu.tr
[1]Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary, University of Balıkesir, Balıkesir, Türkiye
[2]Department of Animal and Dairy Science, University of Wisconsin, Madison, USA
[3]Department of Pathology, Faculty of Veterinary, University of Balıkesir, Balıkesir, Türkiye

Abstract

This study set out to compare the addition of butyrate (sodium-calcium butyrates), herbal extract (*Macleaya cordata*), and yeast (*Saccharomyces cerevisiae*) feed additives on the broiler's growth performance, serum parameters, organ weight, and ileum morphology in the broiler. A total of 320 chicks seven days old were randomly allocated into 4 groups and the trial lasted until 35 days old. Each experimental group comprised four replicate pens (20 chicks per pen). The groups consisted of the basal diet (control group, no additive - C); basal diet + butyrates group (0.5 g/kg sodium-calcium butyrates - UG); basal diet + herbal extract group (0.2 g/kg *Macleaya cordata* - FP), and basal diet + yeast group (1 g/kg *Saccharomyces cerevisiae* - SC). Results showed no significant difference between groups for daily weight gain (DWG), feed intake (FI), and feed conversion ratios (FCR) ($P > 0.05$). However, the body weight gain (BWG) of the FP group was higher than the UG and SC groups at the 21 days ($P = 0.047$). At the end of the study, it was found that the SC group's total antioxidant status level (TAS) was higher than that of the UG and FP groups ($P = 0.033$). It was found that crypt depth was higher in the feed additive groups than in the control ($P < 0.001$), however, villus/crypt ratios were highest in the C and FP groups ($P < 0.001$). In conclusion, 0.2 g/kg herbal extract (*Macleaya cordata)* in broiler diets positively effects on weight gain and villus surface area.

Article highlights

- Herbal extract (*Macleaya cordata*) increased body weight gain in broiler chickens on day 21.
- When yeast (*Saccharomyces cerevisiae*) was added to broiler chicks, the serum total antioxidant status (TAS) and glucose level (GLU) increased.
- The addition of butyrate (sodium-calcium butyrates), herbal extract, or yeast to broiler diets improved the ileum morphology of the chicks.

Keywords Broiler, Butyrate, *Macleaya cordata*, *Saccharomyces cerevisiae*, Serum, Ileum villus/crypt

1 Introduction

Poultry is one of the main sources of animal protein around the world. Antibiotic residue in meat products, disease resistance, and other adverse effects have been linked to the long-term use of antibiotics in chicken production [8, 10]. In 2006, feed additives with different active ingredients were started to be produced instead of these matters, which were removed because antibiotic and hormone applications in feed create residues in foods. Among these feed additives, herbal extracts, prebiotics, probiotics, phytobiotics, yeasts, and synthetic substances have been used [2, 16, 53]. It is estimated that these feed additives used have positive effects on organs such as performance, gastrointestinal system (GIS), microflora, immune system, and endocrine system.

A main requirement for enhancing development performance in poultry is to enhance antioxidant activity because there is a negative correlation between performance and immunity [52]. *Macleaya cordata*, belongs to the *Papaveraceae* family, has a broad spectrum of biological activities such as anti-inflammatory, antibacterial, and antifungal effects [31]. Dietary addiction with 150 mg/kg *Macleaya cordata* increased feed intake, body weight gain, and decreased intestinal lesions of broilers with necrotic enteritis [50]. Previous studies have shown that it has beneficial biological activities, improved the growth performance and intestinal barrier of broilers [30, 34], however, there is little literature evaluating its comparative effects on growth performance and ileum morphology in broiler chickens. In the other side, the hindgut segment produces butyric acid, a short-chain fatty acid, by the enzymatic breakdown of proteins and carbohydrates by probiotic bacteria [35]. Following intestinal absorption, it is transported through the portal vein to the liver, where it is converted into energy-producing ketone bodies [49]. Butyric acid could give intestinal epithelial cells the natural energy they need to proliferate and repair damage [29, 43]. While calcium butyrate is thought to be a source of energy for the enterocytes, which leads to better development of the intestinal villi, sodium butyrate can be used as a green additive in place of antibiotics to improve intestinal health and growth performance by promoting gastrointestinal development in poultry [29, 56]. Furthermore, it is well known that calcium butyrate lowers the pH of the gut mucosa, which fosters the proliferation of healthy commensals in an acidic environment [29]. The last feed additive, *Saccharomyces cerevisiae* (SC), the most common yeast used as a feed additive, has globally gained popularity, recently [9]. This product improves feed conversion, increases bird growth, reduces morbidity, and increases body weight gain [9]. *Saccharomyces* can beneficially modulate the intestinal microflora and epithelial microarchitecture of chickens and help to increase lactic acid bacteria colonization in their gut [9]. It can also support nutrient absorption by increasing the height of the villus and the depth of the crypt [3]. Yeast cells may also help increase immune system function by regulating the expression of enzymatic and non-enzymatic antioxidants in poultry [19].

In the modern poultry sector, where rapid growth rate is frequently associated with increased stress and immune deficiencies, natural growth stimulants could modulate the intestinal permeability of broilers and regulate intestinal morphology, thereby improving immune function and stress resistance. Furthermore, it is estimated that commercial broiler chicks would be slaughtered in fewer than 40 days in the near future. This 35-day study also assessed the impact of natural growth stimulants. These natural growth stimulants feed additives such as butyrate, *Macleaya cordata*, and *Saccharomyces cerevisiae*

could reduce pathogenic microbial loads in the intestine and improve digestion and absorption of nutrients, leading to enhanced animal growth performance. However, their preferences might vary depending on the demands and environment. Therefore, the study aimed to explore the comparative effects of the use of plant extract (*Macleaya cordata*), butyrate (sodium-calcium butyrate), or yeast (*S. cerevisiae*) on growth performance, the weight of organs, some blood parameters, and ileum morphology in broiler chickens.

2 Material and methods

2.1 Ethical approval

Before beginning the study, the ethic certificate was approved by the Ethical Committee of the University of Balikesir, Turkey (Approval number: 8–3/2021).

2.2 Animals, diets, and experimental design

A total of 320 seven-day-old male Ross 308 chicks, which were purchased from a hatchery (Karahallılar Hatchery, Balikesir, Turkiye), were randomly distributed to four treatment groups, with four replicates of twenty chicks. All birds were fed with a starter broiler diet (23% crude protein and 3000 kcal ME/kg) from one day to seven days old. The chicks took one of the following basal diets formulated for each feeding period considering their nutrient requirements between 7 and 35 d National Research Council [37]. In addition, the diets were prepared as isocaloric and isonitrogenous. Experimental groups (Table 1 were formed as; I a basal diet (control, no additive-C group, II basal diet + sodium-calcium butyrate's [UG – 0.5 g/kg Ultraguard Duo, (Turkiye)], III) basal diet + herbal extract (*Macleaya cordata*) [FP – 0.2 g/kg Filopower, (Yem-Vit A.Ş., Turkiye)], and IV) basal diet + yeast [SC – 1 g/kg *Saccharomyces cerevisiae*, (Biosprint, Italy)]. The herbal extract included wheat middlings 50%, a blend of flavoring compounds (magnolia, macleaya) 24%, calcium carbonate 23.5%, products and by products of tubers and roots 2%, and barley meal 5% (units given by producer). The level of use of herbal extract in the study was determined according to Çetin et al. [12] and [38]. The trial period was from 7 to 35 d. All chicks were raised on a 100 × 300 cm wooden

Table 1 Diet composition and nutrient contents of the basal diet (as dry basis)

Ingredients (%)	d 0–21	d 22–35	Nutrient composition (Calculated), g/kg	d 0–21	d 22–35
Corn	53.20	58.80	ME kcal/kg[1]	3007	3191
Soybean meal, 48%	35.47	27.60	Crude protein	226.0	193.0
Vegetable oil	3.00	5.00	Calcium, %	0.96	0.79
Wheat bran	4.00	5.00	Available phosphorus, %	0.48	0.43
Dicalcium phosphate	1.77	1.52	Ether extract	47.7	68.3
DL-Methionine	0.35	0.26	Crude ash	62.2	56.3
L-Lysine	0.27	0.18	Crude cellulose	30.1	29.2
L-Treonin	0.14	0.08	Dry matter	901.0	902.0
Calcium carbonate	1.10	0.86	Nutrient composition (Analyzed)		
Sodium chloride	0.35	0.35	Crude protein, %	22.50	19.10
Trace mineral premix	0.10	0.10	Ether extract, %	4.50	6.67
Vitamin premix	0.25	0.25	Crude ash, %	6.34	5.82
Total	100.00	100.00	Crude fiber, %	4.20	3.20

Vitamin and trace mineral premix; supplied per kg; vitamin D3: 5.000 IU, vitamin E: 30 IU, vitamin A: 12.000 IU, vitamin B1 (thiamin): 3 mg, vitamin B3 (niacin): 45 mg, Manganese: 100 mg, Iron: 60 mg, Zinc: 60 mg, Copper: 5 mg, Cobalt: 0.3 mg, Iode: 1 mg, Selenium: 0.20 mg

[1]Calculated

shavings floor in a temperature and ventilation-controlled room with an 18 h lighting period. All chicks were given ad libitum nipple system and diets throughout the period.

2.3 Feed chemical analysis

Chemical analysis of feed samples were analyzed according to AOAC [7] methods; ether extract (EE, AOAC #920.39), crude protein (CP, AOAC #954.01), crude ash (CA, AOAC #942.05), and crude fiber (CF, AOAC #978.10) (Table 1).

2.4 Sample and data collection

At the end of the experiment, chicks from each replicate similar to the average body weight of the group were randomly selected, weighed, and slaughtered by decapitation. Blood samples were collected from the jugular vein for biochemical analysis, then centrifuged (3000×g for 15 min). The serum sample of blood was immediately stored at – 20 °C. After determining internal organ weight, ileum samples were subsampled to determine measurements.

2.5 Measurement of growth performance

The body weights (BW) of the chickens on days 7, 14, 21, 28, and 35 were weighed, and feed intake (FI), daily weight gain (DWG), and feed conversion ratio (FCR) between periods were calculated. There were no deaths during the trial. The bird's feeding intake was recorded daily.

2.6 Detection of organ weight

The broilers were weighed before slaughtering for blood and organ analysis. Internal organs (liver, heart, gizzard, proventriculus, spleen, and intestine) were attentively weighed. These weights were calculated by dividing these traits by the final body weight.

2.7 Serum biochemical analysis

At the end of the experiment, total antioxidant status (TAS) levels were determined using the automated measurement method (Rel Assay Diagnostics Kit, Mega Tip, Gaziantep, Turkey) [18]. The novel automated method is based on the bleaching of the characteristic color of a more stable ABTS (2,2 ′- Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation by antioxidants. Total protein (TP), albumin (ALB), serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), uric acid (UA), low-density lipoprotein (LDL), high-density lipoprotein (HDL), glucose (Glu), and triglyceride (TG) was measured with the aid of a biochemical autoanalyzer (Mindray, BS300, Shenzhen, China).

2.8 Histopathological examinations

Tissue samples from the ileum were collected in 10% neutral buffered formalin. After the fixation, samples were dehydrated alcohol, cleared in xylene, and embedded in parafine. Then, 4 µm sections were cut from the parafine bloks and stained Hematoxylin–Eosin. Histopatological changes were examined under a light microscope (Nikon, Eclipse Ni, Tokyo, Japan) [44]. The villi height (VH, µm) was determined from the apex of the villi to the Lieberkühn crypts, and the crypt depth (CD, µm) was measured as the depth of invagination between the villi.

2.9 Statistical analysis

A completely randomized design with four groups and four replicates was used in this study. The normality of the variables was tested with the Shapiro–Wilk test. All statistical analyses were carried out using SPSS, version 25.0 (SPSS Inc., Chicago, IL, USA). Data were compared among dietary treatment groups using a One-Way ANOVA followed by the Tukey Multiple Comparison Test.

3 Results

As shown in Fig. 1, an increase in BW was detected in the herbal extract (FP) supplemented group at d 21 ($P = 0.047$); but it was not significant on d 35 ($P > 0.05$). The DWG of broilers was not significant in all periods ($P > 0.05$). Likewise, the overall FI and FCR of broilers were not significant ($P > 0.05$).

The effects of herbal extract, butyrate, or yeast on blood parameters are presented in Fig. 2. The TP, AST, ALT, HDL, TG, and UA were not affected by using these feed additives ($P > 0.05$). The addition of SC significantly increased TAS (1.98 mmol/L) and GLU (294.83 mg/dL) ($P < 0.05$). The lowest LDL (5.81 mg/dL) was found in the UG, whereas the highest was determined in FP (10.83 mg/dL) ($P = 0.012$).

In this study, measurements of organ weights (g/100 g BW) are given in Fig. 3. Liver weight was found to be lower in the FP compared to the C, however, it was not significant ($P = 0.100$). The intestinal weight in UG was higher than the C ($P = 0.033$).

Results for the morphological measurement of the ileum are given in Fig. 4. There were no differences among the groups in terms of villus length, while the crypt depth was found to be 594.81, 1131.95, 926.56, and 1112.24 µm in the C, UG, FP, and SC groups, respectively ($P < 0.001$). The villus/crypt ratio of the groups was 4.111, 2.401, 3.747, and 2.415, respectively. The highest villus/crypt ratios were found in the C and FP groups ($P < 0.001$).

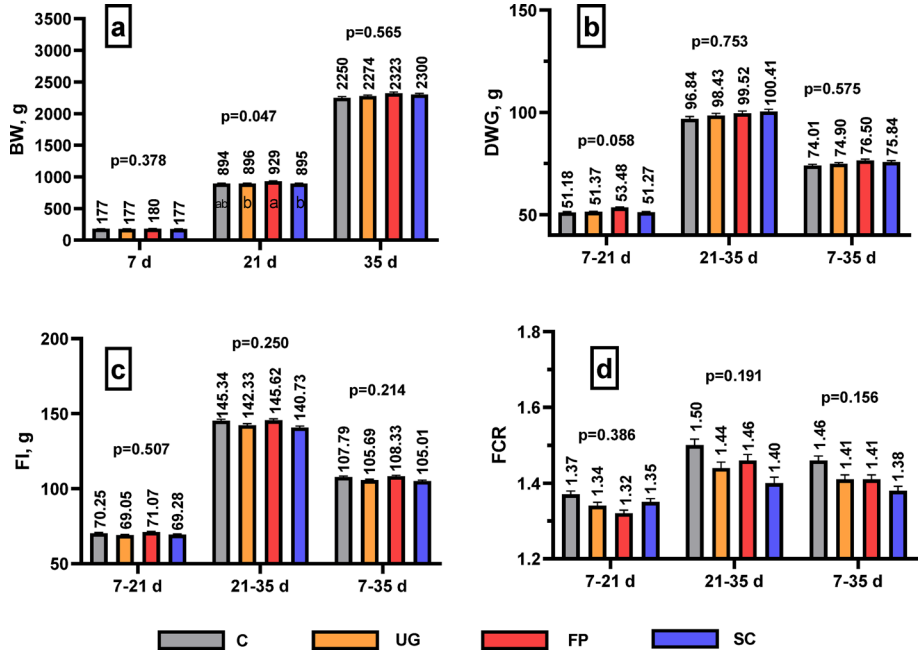

Fig. 1 Broiler growth performance parameters. BW: Body weight, DWG: Daily weight gain, FI: Feed intake, FCR: Feed conversion ratio. C: Control, UG: Sodium-calcium butyrate additive, FP: *Macleaya cordata* additive, SC: *Saccharomyces cerevisiae* additive

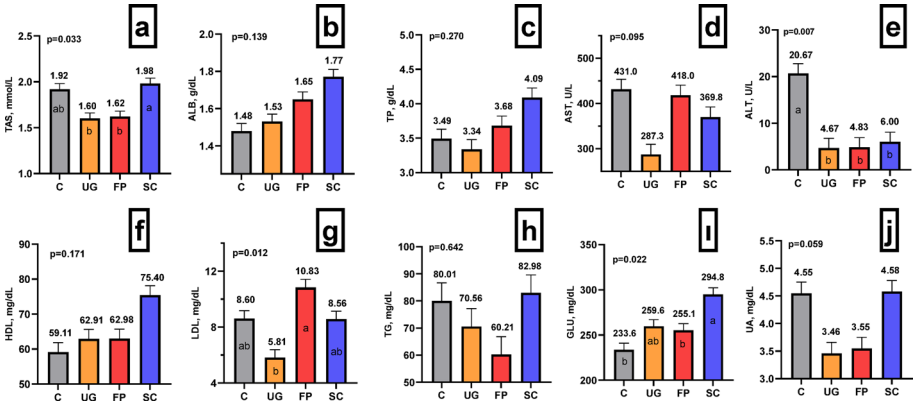

Fig. 2 Serum biochemistry parameters. TAS: Total antioxidant status, ALB: Albumin, TP: Total protein, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, TG: Triglyceride, Glu: Glucose, UA: Uric acid. C: Control, UG: Sodium-calcium butyrate additive, FP: *Macleaya cordata* additive, SC: *Saccharomyces cerevisiae* additive

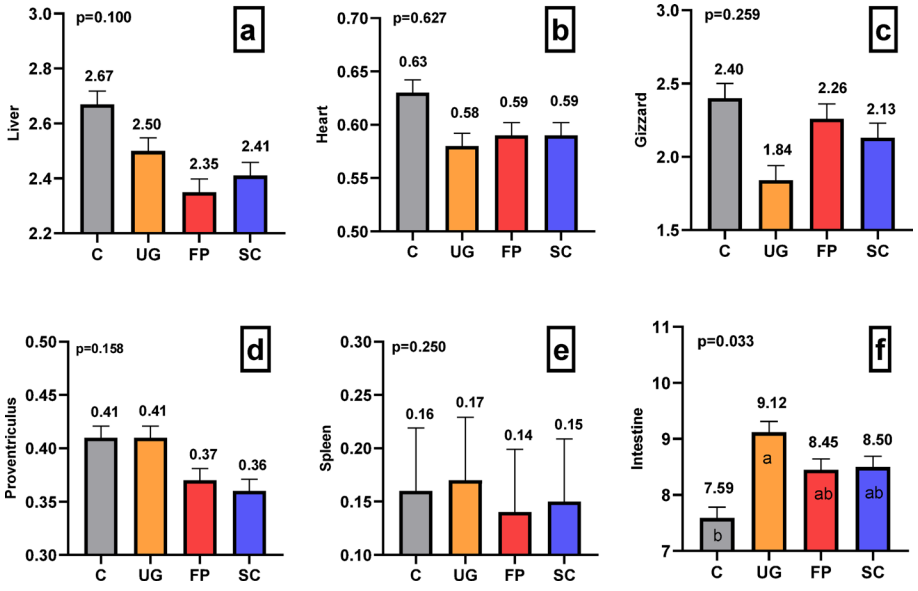

Fig. 3 Internal organ weights, g/100 g BW*. *Organ weight (g/100 g body weight) of sampled chicks. C: Control, UG: Sodium-calcium butyrate additive, FP: *Macleaya cordata* additive, SC: *Saccharomyces cerevisiae* additive

4 Discussion

In broilers, various feed additives to increase growth performance provide benefits by affecting growth and feed digestibility through different mechanisms [5, 16, 41]. Broiler's growth performance in terms of some parameters within the growth period is linked to intestinal morphology, such as various other factors, including management, environment, and breeding. In the current study, the different effects and comparisons of adding butyrate, *Macleaya cordata*, and *Saccharomyces cerevisiae* on diets were investigated on growth performance, serum biochemistry, organ weights, and ileum morphology of broilers. In the present study, the acquired results showed that a beneficial effect of natural growth stimulants in addition to the broiler diets on DWG, FI, and FCR were evaluated and no significant changes among the experimental groups were found. However, the findings clearly show that herbal extract supplementation significantly increased

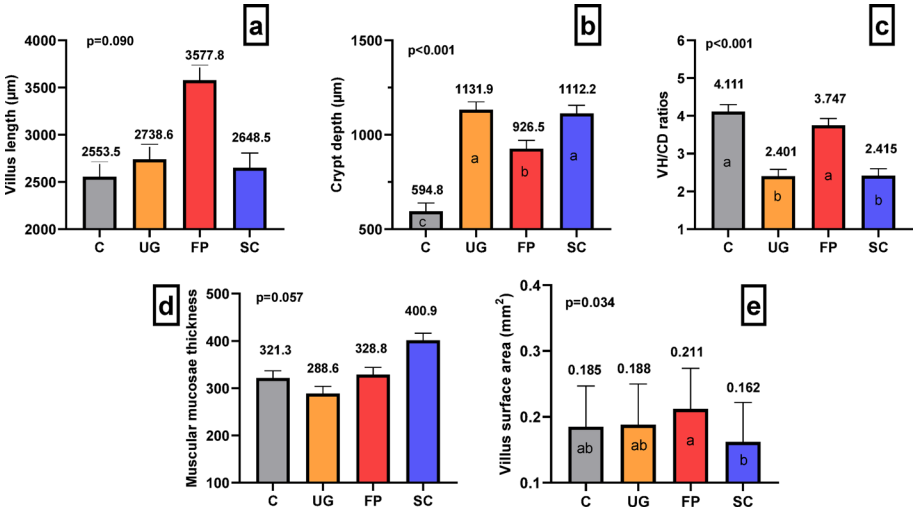

Fig. 4 Ileum villus, crypt, muscular mucosae, and surface measurement (µm). VL/CD ratios: villus length/crypt depth ratios. C: Control, UG: Sodium-calcium butyrate additive, FP: *Macleaya cordata* additive, SC: *Saccharomyces cerevisiae* additive

body weight gain on 21-day. Kozlowski et al. [28] reported that the *Macleaya cordata* (plant from which sanguinarine is extracted) additive in the diet had no significant effect on feed intake, body weight gain, or feed conversion rate. In the study by Vieira et al. [46], in which *Macleaya cordata* extract (Sanguinarine) and organic acid were added to broiler diets, it was reported that there was an increase in body weight at 14 and 21 days compared to the control. In some studies, it was stated that the addition of plant extracts and essential oils to the diets produced a very high weight in chickens and had a growth-promoting effect [13, 24]. Similarly to our results, Vieira et al. [46] reported that there were no different effects of the addition of these extracts to the broiler diets. The impact of feed additives on live weight could change according to breed, ambient factors, and ration composition.

Serum biochemical could be a health indicator as a means of verifying the health conditions of chickens and could reveal potential alterations in the physiological system depending on the effects of environment, feeding, management, and stress level [11, 26]. These parameters indicate the metabolism of nutrients in the body [32]. In the study, it was observed that the TAS levels of the SC group increased compared to the UG and FP groups, which is consistent with Fathima et al. [19], who reported that yeast increases TAS levels. TAS represents the antioxidant capacity of defense mechanisms and measures the body's antioxidant capability [54, 55]. Yeast modulates the host's immune system through various immune-competent cells [36]. *Saccharomyces cerevisiae* could reduce oxidative stress by neutralizing free radicals, which can lead to an increase in antioxidant enzyme levels in blood serum [27]. Yeast is a feed additive containing high levels of non-starch polysaccharides, mannose, and glucan [23]. It was observed that glucose, which is the main energy source for the body, increased in the SC group compared to the control. Direct fed microbials could improve glucose metabolism, which can help regulate blood sugar [15]. It specifically recognizes many mannose-containing carbohydrates characteristics of microorganisms and mediates their phagocytosis by macrophages [21]. This may be related to the macrophage mannose receptor and endocytic pattern recognition receptor [36]. Generally, LDL is responsible for transporting

triglycerides and total cholesterol from the liver to peripheral tissues, while HDL is responsible for transporting total cholesterol from extrahepatic tissue to the liver and is crucial for the processes of anti-inflammatory, anti-apoptotic, and antioxidant properties [34]. Recent studies have claimed that blood lipids play a significant role between cholesterol levels and immunological changes [42, 45]. A report found a strong correlation between certain immune cell subpopulations and HDL cholesterol concentrations [42]. In this study, the increasing trend of HDL in the SC group among the groups shows that it could have an effect on the TAS level. As LDL accumulates in the intima, it activates the endothelium to release chemokines and leukocyte adhesion molecules, which in turn lead to the activation of signaling pathways and the production of inflammatory cytokines [47]. Acute tissue damage and chronic disease both exacerbate necrosis or apoptosis due to the excess secretion of inflammatory cytokines, which are essential for the host's defense against pathogens and response to them [14]. This situation could be connected to the plant extract's beneficial effects on 21-day BW as well as VL/CD and villus surface area on the ileum at the end of the study. We found that natural growth stimulant supplementation decreased serum ALT activity in this study. A common enzyme in hepatocytes, ALT has an activity that is roughly 3,000 times higher in the liver than in serum [14]. Additionally, serum ALT activity is frequently employed as a clinical detection technique and a significant signal to assess hepatocyte injury in the body. ALT will be released into the bloodstream when the liver is damaged, and its increased frequently indicates hepatocyte (cellular) injury [34]. In this study, all-natural growth stimulants had a positive effect on ALT values.

When organ weights were evaluated, liver weight in the control group tended to increase compared to the other groups. An increase in liver weight in birds treated with natural growth stimulants is indicative of hyperplasia of the parenchyma or bile ducts, especially if this increase is not accompanied by a higher BW compared to the control group of birds [4]. Plasma aspartate aminotransferase (AST) levels in control diets were higher compared to other diets and as an indicator of liver damage, we speculate that an inflammatory process in the liver might be responsible for the increased weight. However, Çetin et al. [12] added FP to broilers' diets and found an increase in liver weight. When the intestinal weight was evaluated, it was observed that the combined treatment of calcium and sodium butyrate had an increasing effect on the intestinal relative weight. This situation was similar to Wu et al. [48]. It is possible that enterocytes preferentially mediate the effect of butyrate on intestinal weight in broilers to promote growth and function.

The effectiveness of the use of natural growth stimulants in poultry is partly dependent on their digestive properties [51]. The small intestine, especially the absorptive epithelial villi and crypts, plays a crucial role in the final stage of digestion and integration of nutrients [6]. Measurement of the length, depth, and surface area of crypts and villi can be used to assess the efficiency of digestion and absorption [6, 20]. It has been reported that there are some changes in the morphology in the intestine and increasing digestive enzyme secretions by stimulating primarily pancreas exocrine activity according to the diet type [1, 25, 39]. In the present study, histomorphological examinations of the intestine, the treatment groups had higher values in terms of crypt depth compared to the control group. In studies on crypt depth, positive effects of yeast [40], butyrate [29], and sanguinarine [33] have been reported. In our study, although the villi lengths were not

significant, they were numerically higher in the ileum in the sanguinarine group compared to the others. These findings may be related to the lower pH of the mucous morphology by the sanguinarine [33, 50]. Our results indicated that dietary supplementation with combined butyrate, herbal extract, and yeast played a beneficial role in improving the ileum morphology of the broiler. It was observed that villus surface SC was lower than FP. In this study, GIS samples from chickens fed SC were observed to have thicker muscularis mucosa. The muscular mucosa is a thin layer of smooth muscle at the border between the gastrointestinal mucosa and submucosa, and the reduced thickness may be associated with the presence of undesirable bacteria [17, 22].

5 Conclusion

This study shows that the use of herbal extract (*Macleaya cordata)* in diets in broilers has positive effects on body weight gain, LDL level, and villus surface area. On the other hand, it is seen that the use of yeast in the diets of broilers can improve the antioxidant status. Moreover, combined butyrate could affect crypt depth. These new pieces of information could be able to benefit from antibiotic-free natural animal production. It needs to be conducted new research comparing the effects of the feed additives of the growth of crypts and villi in the intestine with how they affected weight gain and microbial development.

Author contribution

MZ: Formal analysis, Investigation, Data curation, Methodology, Visualization, Writing – original draft, Writing – review & editing; ED: Validation, Investigation, Funding acquisition, Supervision, Methodology, Writing – review & editing; MU: Formal analysis, Methodology, Investigation, Writing – review & editing; OKB: Formal analysis, Investigation; MK: Methodology, Validation; MAA: Conceptualization, Supervision.

Funding

This study was supported by the Scientific Research Projects Coordination Unit of Balıkesir University (Project #: 2021-142).

Data availability

The data is available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was conducted based on the permission of the Balıkesir University Animal Experiments Local Ethics Committee (Approval number: 8–3/2021).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 7 March 2025 / Accepted: 17 July 2025

Published online: 11 August 2025

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